# STUDIES ON THE CONTROL OF DEVELOPMENT. DIFFERENCES IN THE PATTERN OF DNA BINDING PROTEINS ISOLATED FROM VEGETATIVE AND SPORULATING CELLS OF *BACILLUS SUBTILIS*

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## 1. Introduction

The conversion of vegetative cells of *Bacillus subtilis* into spores is a delicate process associated with a number of drastic changes in macromolecular synthesis (for review see ref. [1]). DNA synthesis is turned off at or shortly after $T_0$ [2]. Net RNA and protein synthesis also stops, but RNA and protein turnover is observed for quite some time after $T_0$ in sporulating cells [3].

These changes can be induced by changes in the environment of vegetative cells, for example by starvation for amino acids and glucose [4]. However, the mechanism of this induction is unknown. We have proposed recently that regulatory nucleotides (HPN) are involved in this process [5] and found that HPN I (adenosine tetraphosphate) and HPN II (adenosine pentaphosphate) are produced by ribosomes from sporulating cells exclusively whereas ribosomes from vegetative cells synthesize MS I and II [6]. MS nucleotides seem to regulate transcription of certain genes in *E. coli* [7] and possibly in vegetative cells of *B. subtilis* [8].

If HPN has similar functions in sporulating *B. subtilis* cells, then one would expect different proteins involved in transcription of sporulation specific genes. Since proteins engaged in transcription are expected to have a high affinity to DNA, we have investigated the pattern of DNA binding proteins from vegetative and sporulating cells and indeed found a number of characteristic differences.

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## 2. Material and methods

### 2.1. *Bacterial strain*

*B. subtilis* strain 60015 (ind$^-$, met$^-$), the transformable Marburg strain (= SB 26 of Nester) was used throughout these studies.

### 2.2. *Media and growth conditions*

The SYM medium used in this study as well as the conditions for growth and sporulation have been described in detail previously [9].

### 2.3. *Preparation of crude extract*

To 100 ml SYM medium with the amino acids contained in yeast extract rather than yeast extract itself except methionine and tryptophan which was present as [$^3$H] methionine (250 $\mu$Ci, spec. act. 100 Ci/mol) and [$^3$H] tryptophan (250 $\mu$Ci, spec. act. 4.5 Ci/mmol) $10^6$ cells were added and incubated at 37°C in a shaker water bath. After 60 min the concentration of methionine and tryptophan was increased to give a final concentration of 2 $\mu$g/ml and 5 $\mu$g/ml, respectively. For the preparation of DNA binding proteins protease inhibitors like diisopropyl-fluorophosphate (DEP, 1 mM), phenylmethylsulfonyl fluoride (PMSF, 1 mM) and EDTA (5 mM) were added at $A_{600\ nm} = 0.8$. DNA binding proteins were grown in SYM medium and the protease inhibitors were added at $T_{2.5}$. Short-

ly thereafter, cells were centrifuged at 15 000 *g* for 10 min. The pellet was washed twice with 0.05 M Tris–HCl buffer pH 7.4 containing 1 M KCl and inhibitors. The cells were resuspended in buffer, containing 20 mM Tris HCl pH 7.4, 2 M NaCl, 1 mM mercaptoethanol, 5 mM EDTA, 10 mM DFP and 1 mM PMSF, and disrupted in a French Press. After removal of cell debris by centrifugation the supernatant was treated as described by Alberts et al. [10] using PEG precipitation.

2.4. *DNA cellulose chromatography*

DNA cellulose was prepared as described [11] as was the preparation of DNA-binding proteins, which were eluted with 2 M NaCl.

2.5. *SDS gel electrophoresis*

DNA-binding proteins ($5 \times 10^4$ cpm) were subjected to SDS gel electrophoresis as described previously [12].

The entire gel was then cut in 1 mm slices and dissolved in 0.5 ml 0.5 M NaOH by incubation at 32°C overnight. Then 6 ml of Bray's solution containing 20 g/litre Cab-o-sil were added and radioactivity was counted in a Packard Scintillation spectrometer.

## 3. Results and discussion

The isolation of DNA binding proteins has been used in many cases to characterize substances associated with DNA replication or regulation of gene expression (for review see ref. [10]). Since regulation of sporulation also involves processes like replication, transcription etc. we wanted to investigate whether or not this process also involves regulating proteins with affinity to DNA. We therefore isolated DNA-binding proteins from vegetative cells and also sporulating cells of *B. subtilis*. Any differences in the patterns of DNA-binding proteins from the different stages of development could indicate that proteins participate in the regulation of sporulation.

One essential precaution during isolation of DNA-binding proteins is the complete inhibition of any protease activity present in *B. subtilis* cells, especially in sporulating cells. Numerous experiments with mutants that produce reduced amounts of proteases and with protease inhibitors (data not shown) revealed that DFP, PMSF, EDTA together with low temperature can completely inhibit protease activity during the isolation procedure.

We are therefore confident that differences in the pattern of DNA-binding proteins isolated from different stages during the *B. subtilis* cell cycle are not artefacts caused by protease activity.

In order to increase the sensitivity for the detection of different regulatory proteins, we labeled all proteins of vegetative and sporulating cells heavily with [$^3$H]methionine and [$^3$H]tryptophan necessary for growth of our strain and isolated DNA-binding proteins as described by Alberts et al. [10]. Approximately 2–3% of all proteins of vegetative cells can bind to DNA cellulose. Sporulating cells contain between 0.8 and 1.5% DNA binding proteins.

Of the total amount of soluble proteins applied to cellulose 0.1–0.3% are unspecifically bound. Approximately 2–4% of DNA binding proteins can not be eluted by a 2 M NaCl rinse.

When proteins isolated from vegetative cells which bind to DNA cellulose were eluted with 2 M NaCl and subjected to SDS gel electrophoresis, the following pattern was obtained (see fig. 1, solid line). A number of proteins with different molecular weight seem to have affinity to DNA and can therefore be regarded as regulatory proteins or enzymes involved in replication, transcription, repair etc. We have not yet determined which proteins have either one of these functions nor are data available from other laboratories.

More important, however, at present are differences in DNA-binding proteins isolated from sporulating cells as compared to those from vegetative cells. As can be seen from fig. 1 (dotted line) a number of proteins present in vegetative cells are either missing or additional proteins are detectable. For example at least four proteins with approximate molecular weights between 140 000 and 350 000 present in vegetative cells have disappeared, whereas other proteins with molecular weights of approximately 25 000–40 000 have appeared with a reduced level and new proteins of about 13 000 mol. wt. were found only in sporulating cells.

At the moment, we cannot clearly attribute any one of these proteins to specific functions during spore formation. However, this remarkable difference is an indication of the involvement of DNA-binding proteins in the regulation of sporulation.

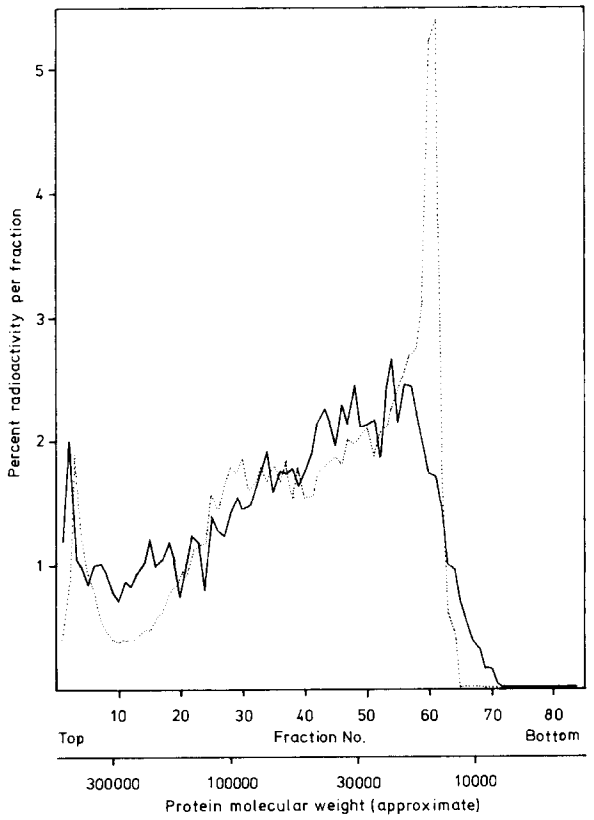


Fig. 1. SDS gel electrophoresis of DNA binding proteins (see Materials and methods) from vegetative cells harvested at $A_{600\ nm} = 0.8$ (solid line) and sporulating cells, harvested at $T_{2.5}$ (dotted line).

Preliminary experiments (data not shown) with DNA binding proteins isolated from cells after $T_0$, which cannot sporulate because of inhibition of sporulation by glucose, show a pattern similar to vegetative cells rather than sporulating cells indicating that indeed some of the proteins present in sporulating cells are involved in sporulation. However, more work is needed to clearly prove this conclusion.

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